

Porcine circovirus type 2 (PCV2) vaccination is effective in reducing disease and PCV2 shedding in semen of boars concurrently infected with PCV2 and *Mycoplasma hyopneumoniae*

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Abstract

The objectives were to determine whether the amount of porcine circovirus type 2 (PCV2) shed in semen increased in boars experimentally coinfecting with *Mycoplasma hyopneumoniae* (MHYO), and whether PCV2 vaccination of boars prior to PCV2 exposure reduced PCV2 viremia and virus shedding in semen. Twelve specific-pathogen-free PCV2- and MHYO-naïve boars were randomly and equally assigned to one of four groups. Six boars were vaccinated against PCV2 (VAC) on Day 0; three PCV2 vaccinated and three non-vaccinated boars were inoculated with MHYO on Day 21, and all boars were challenged with PCV2 on Day 35. The four treatment groups included PCV2-Infected (I), VAC-PCV2-I, MHYO-PCV2-Coinfected (CoI), and VAC-MHYO-PCV2-CoI. Semen, blood swabs, feces, and serum samples were collected weekly until Day 70. All vaccinated boars had seroconverted to PCV2 by Day 35. Between Days 28 and 35, MHYO boars developed moderate respiratory disease, characterized by coughing, respiratory distress, mucopurulent nasal discharge and loss of body condition. One MHYO-PCV2-CoI boar died on Day 50. Boars in the PCV2-I and MHYO-PCV2-CoI groups had significantly higher PCV2 DNA loads in blood swabs than the remaining boars. Moreover, PCV2 vaccination significantly reduced the incidence and amount of PCV2 shedding in semen and feces. In summary, although concurrent MHYO infection did not influence PCV2 shedding patterns, coinfection of boars with PCV2 and MHYO resulted in severe clinical disease and viral shedding was significantly decreased by PCV2 vaccination.

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Keywords: *Mycoplasma hyopneumoniae* (MHYO); Porcine circovirus type 2 (PCV2); Semen shedding; PCV2 vaccination; Pig

1. Introduction

Porcine circoviruses (PCV) are small, single-stranded, circular, non-enveloped DNA viruses

which belong to the family *Circoviridae* [1]. To date, there are two recognized PCV genotypes referred to as PCV type 1 (PCV1) and PCV type 2 (PCV2) [2,3]. Porcine circovirus type 2 can be further divided into several subtypes, of which PCV2a and PCV2b are prevalent worldwide [4]; PCV2c has been detected in Denmark [5], and PCV2d and PCV2e were detected in China [6].

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Porcine circovirus type 1 is non-pathogenic [7,8] whereas PCV2 is associated with several disease manifestations, collectively referred to as porcine circovirus associated disease (PCVAD) [9]. Porcine circovirus associated systemic disease or postweaning multisystemic wasting syndrome (PMWS) [10], and PCV2-associated respiratory disease as a part of the porcine respiratory disease complex [11,12], are widely recognized. One less common manifestation of PCVAD is reproductive failure in breeding females, manifested primarily as more mummified fetuses at term [13], usually without clinical signs in the dam [14].

Porcine circovirus type 2 can be transmitted vertically and horizontally. Although the oronasal route is considered the primary route for transmission of PCV2 [15–17], breeding animals could be infected with PCV2 via semen from infected boars. In that regard, PCV2 DNA was shed in semen of both naturally [18–20] and experimentally infected [21,22] boars. Furthermore, PCV2 DNA in boar semen was infectious in a swine bioassay model [23]. However, it was noteworthy that under experimental conditions, the amount of PCV2 shed in semen was low [22] and was not transmitted to naïve breeding animals via AI [23].

In the growing pig model, *Mycoplasma hyopneumoniae* (MHYO) infection potentiated PCV2 replication, PCV2-associated lesions, and clinical disease [24]. Immunomodulation associated with MHYO infection may potentiate the effects of infection with PCV2 [25]. Under field conditions, young boars that enter boar studs are often exposed to infectious agents such as MHYO for the first time, are given multiple adjuvanted vaccines, and are exposed to other stressors (mixing, transportation) thought to enhance PCV2 replication in growing pigs [26].

The main objective of the present study was to determine whether the amount of PCV2 DNA shed in semen increased in boars experimentally coinfecting with MHYO and immune-stimulated via administration of an inactivated porcine parvovirus-*Leptospira* spp.-*Erysipelothrix rhusiopathiae* (PLE) vaccine. In addition, the effect of PCV2 vaccination of boars prior to PCV2 exposure on PCV2 viremia and virus shedding in semen was also determined.

2. Materials and methods

2.1. Animals and housing

Twelve 9 month old, specific-pathogen-free (SPF) boars were obtained from an experimental research barrier herd that was continuously maintained for sev-

Table 1

Experimental design for this study (PCV2, porcine circovirus type 2; PLE, porcine parvovirus-*Leptospira* spp.-*Erysipelothrix rhusiopathiae*; MHYO, *Mycoplasma hyopneumoniae*).

| Group designation | No. boars | Vaccination | Innocation |
|-------------------|-----------|-------------|------------|
| PCV2-I | 3 | PLE | PCV2 |
| VAC-PCV2-I | 3 | PCV2 | PLE |
| MHYO-PCV2-CoI | 3 | PLE | MHYO |
| VAC-MHYO-PCV2-CoI | 3 | PCV2 | PLE |

eral generations under BSL-3 conditions for more than a decade. This herd was tested regularly and confirmed free of the following pathogens: PCV2, porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), and MHYO as determined by routine serology and PCR surveillance. After arrival, boars were randomly allocated into six rooms (3 × 4 m; two boars per room) in a BSL-2 facility at the National Animal Disease Center (NADC) in Ames, IA, USA. All rooms were identical in size, had a separate ventilation system, and were equipped with a nipple waterer. Once daily, boars were fed a balanced, pelleted, complete feed ration, free of animal proteins and antibiotics (Nature's Made, Heartland Coop, Cambridge, IA, USA).

2.2. Experimental design

The experimental design is summarized (Table 1) and the timeline of events is shown (Fig. 1). The 12 boars were randomly and equally assigned to treatment groups and rooms. Each of the six rooms contained one vaccinated (VAC) and one non-vaccinated boar. Three rooms were used for PCV2 infected (PCV2-I) boars, whereas boars in the remaining three rooms were coinfecting with PCV2 and MHYO (MHYO-PCV2-CoI) with the following group designations: PCV2-I, VAC-PCV2-I, MHYO-PCV2-CoI, and VAC-MHYO-PCV2-CoI. Porcine circovirus type 2 vaccination was done on Day 0, MHYO inoculation was done on Day 21, PLE vaccination was done on Days 21 and 35, and PCV2 inoculation was done on Day 35. Semen, serum, feces, and blood swabs were collected once weekly from Days 35 to 70, and necropsies were done on Day 70. In addition, six sentinel negative control animals (all females) of similar age, breed and origin as the boars, were kept in three additional rooms in the same building under similar conditions as the boars until Day 70. The experimental protocol was approved by both the NADC and Iowa State University Institutional Animal Care and Use Committees (NADC IACUC number 3972 and Iowa State IACUC number 4-09-6725-S).

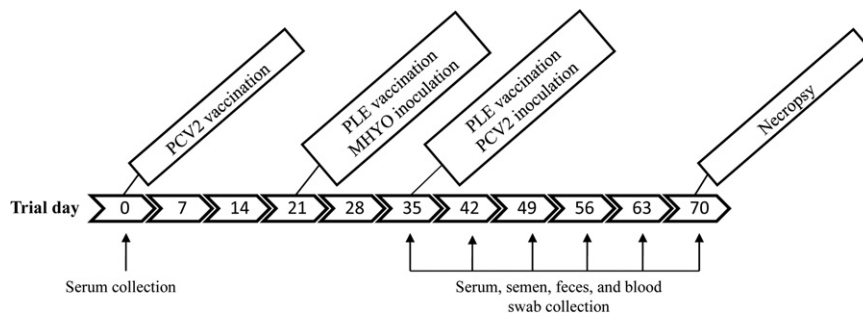


Fig. 1. Timeline of events during current study.

2.3. Clinical evaluation

All boars were evaluated daily for signs of respiratory disease, e.g., sneezing, coughing, nasal discharge, lethargy, and decreased appetite.

2.4. Vaccination

2.4.1. Porcine circovirus type 2

On Day 0, a commercial PCV2 vaccine (Suvaxyn® PCV2, Fort Dodge Animal Health; Fort Dodge, IA, USA; Serial number 1861220A) licensed for pigs ≥ 3 wk of age, was administered according to manufacturer's instructions. Each boar in the VAC-PCV2-I and VAC-MHYO-PCV2-CoI groups was vaccinated with 2 mL of the PCV2 vaccine which contained an inactivated chimeric PCV1-2a (ORF1 of PCV1 and ORF2 of PCV2a), given i.m. in the right neck area.

2.4.2. Porcine parvovirus-Leptospira spp.-Erysipelothrix rhusiopathiae (PLE)

On Days 21 and 35, all boars were vaccinated with 2 mL of a commercial inactivated PLE vaccine (FarrowSure® GOLD, Pfizer Animal Health, Inc.; Exton, PA, USA; Serial number A940661) according to the manufacturers' instructions. The PLE vaccination was given i.m. into the right neck area.

2.5. Inoculation

2.5.1. Mycoplasma hyopneumoniae

A stock lung homogenate (LI42 11-24-08) that contained pig-passaged MHYO isolate 232, a derivate of MHYO strain 11, was used. One tube of stock lung homogenate was thawed in cool water and diluted 1:75 in Friis medium without antibiotics (lot 1527). The diluted MHYO inoculum (10^5 color changing units per mL) was kept on ice and used within 2 h after preparation. On Day 21, each boar in the MHYO-PCV2-CoI and VAC-MHYO-PCV2-CoI groups was inoculated with 30 mL MHYO inoculum, administered in increments of 10 mL, with a 20 min interval between suc-

cessive inoculations. The MHYO inoculum was slowly given intranasally (5 mL per nostril) during inspiration using a 12 mL syringe (Fisher Scientific Inc.) and 1.3 \times 48 mm catheter (Benton Dixon Infusion Therapy Systems Inc., Sandy, UT, USA).

2.5.2. Porcine circovirus type 2

PCV2b strain NC16845 [27], approximately $10^{4.5}$ 50% tissue culture infectious dose (TCID₅₀) per mL, was used for inoculation. On Day 35, all boars were intranasally and intramuscularly inoculated with PCV2b. Inoculation was done by slowly dripping 2.5 mL of the inoculum in each nostril (5 mL in total) and injecting 2 mL i.m. into the left neck area.

2.6. Sample collection

Once weekly from Days 35 to 70, semen, serum, blood swabs and feces were collected. The metal dummies used for the semen collection were located in separate collection pens. Semen collections were done by a gloved-hand technique, using an insulated container lined with a 2 L disposable plastic bag and tear-away filter. Collection bags containing raw semen were gently agitated to resuspend sperm equally. During semen collection, serum, blood swabs and feces were collected while the boar was mounted on the collection apparatus. Whole blood was collected into a 300 μ L serum gel capillary tube (Safe-T-Fill®, RAM Scientific Inc., Yonkers, NY, USA) by venipuncture of an ear vein. Similarly, blood swabs were collected using individually packaged sterile swabs (QuickVue® In-Line Strep A swabs, Quidel Corp., San Diego, CA, USA) and placed into a sterile 5 mL polystyrene round bottom tube (Falcon®, BD Biosciences, San Jose, CA, USA) containing 1 mL of sterile saline solution (Fisher Scientific Inc.). A fecal sample was collected directly from the rectum and inserted into a 5 mL polystyrene round bottom tube (Falcon®, BD Biosciences). All samples were taken immediately to the laboratory. Ali-

quots of raw semen were pipetted into 1.5 mL sterile tubes, blood was centrifuged at $2000 \times g$ for 10 min at 4 °C, and serum separated. All samples were stored at –80 °C until testing.

2.7. Serology

2.7.1. Porcine circovirus type 2

Serum samples collected on Days 0, 35, 42, 49, 56, 63, and 70 were tested with a PCV2 open reading frame (ORF)-2 based, in-house ELISA, as previously described [28]. A sample-to-positive (S/P) ratio of ≥ 0.2 was considered positive.

2.7.2. *Mycoplasma hyopneumoniae*

Serum samples collected on Days 0, 35, and 70 were tested by ELISA as described [29]. Known positive and negative sera were included on the plate. Optical density (OD) readings >2 SD above the mean value of the negative control were considered positive.

2.8. Detection and quantification of PCV2 DNA

Deoxyribonucleic acid extraction was performed using the QIAamp® DNA Mini Kit (Qiagen, Valencia, CA, USA). Deoxyribonucleic acid extracts were used for quantification of the PCV2 genomic DNA copy numbers by real-time PCR, as previously described [30], using ORF1-based primers and a probe. The PCR reaction was done in 25 μ L PCR mixtures that contained 12.5 μ L of a commercially available master mix (TaqMan Universal PCR Master Mix, Applied Biosystems Inc., Foster City, CA, USA), 2.5 μ L DNA extract, 1 μ L forward and reverse primers, 0.5 μ L detection probe with concentrations of 10 μ M, and 7.5 μ L water. On each plate, five progressive 1:10 dilutions of a known copy number of PCV2 genomic DNA excised from a purified PCV2 DNA clone, were included to generate a standard curve. Each plate was run in the sequence detection system (7500 Sequence Detection System; Applied Biosystems Inc.) under the following conditions: 2 min at 50 °C, 10 min at 95 °C, followed by 38 cycles of 15 s at 95 °C, and 1 min at 60 °C. Samples that did not generate a signal following 38 cycles were considered negative.

2.9. Necropsy

On Day 70, boars were humanely killed with an overdose of pentobarbital (Vortech Pharmaceuticals, Dearborn, MI, USA) and necropsied. Macroscopic lung lesions, scored from 0 to 100% of the lung affected, and the size of lymph nodes, scored from 0 (normal) to 3 (four times the normal size), were graded in a blinded

fashion, as described previously [31]. Sections of lymph nodes (superficial inguinal, mediastinal, tracheobronchial, and mesenteric), lung, tonsil, thymus, ileum, kidney, colon, spleen, liver, testis, epididymis, prostate, bulbourethral gland, and seminal vesicle were collected, fixed in 10% neutral-buffered formalin, and routinely processed for histological examination.

2.10. Histopathology and immunohistochemistry for PCV2

Histopathology slides of all organ systems were scored individually in a blinded fashion by a veterinary pathologist (TO) for the presence and severity of lesions. Severity of lymphoid depletion and the presence and severity of lymphohistiocytic inflammation were scored as previously described in our model [24]. Immunohistochemistry (IHC) for PCV2 [32] was done on selected tissue sections (lymphoid tissues and sex glands), and the amount of virus antigen was estimated and scored (0 = no virus detectable, 3 = large amounts of virus).

2.11. Statistical analyses

Summary statistics were calculated for each group cross-sectionally, to assess overall data quality. Serological and viremia data were analyzed using a repeated measures analysis of variance (ANOVA) to test the null hypothesis that there was no effect of time on differences between samples. The sample type (serum, blood swab, feces, and semen) was the fixed, independent variable, whereas continuous data (\log_{10} PCV2 genomic copies per mL or PCV2 ELISA S/P ratios) were dependent variables. If there was a significant effect ($P < 0.05$) in the repeated measures ANOVA, a one-way ANOVA was performed at each trial day. The rejection level for the null hypothesis was 0.05, followed by pairwise testing using the Tukey-Kramer adjustment. A non-parametric ANOVA (Kruskal-Wallis) was used for data that were not normally distributed data, or when group variances were dissimilar; for these analyses, pair-wise comparisons were done using a Wilcoxon rank sum test. Statistical analysis was performed using JMP® software, Version 8.0.1 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Clinical disease

Between Days 28 and 35 (following inoculation with MHYO), infected boars developed moderate respiratory disease, characterized by coughing, respi-

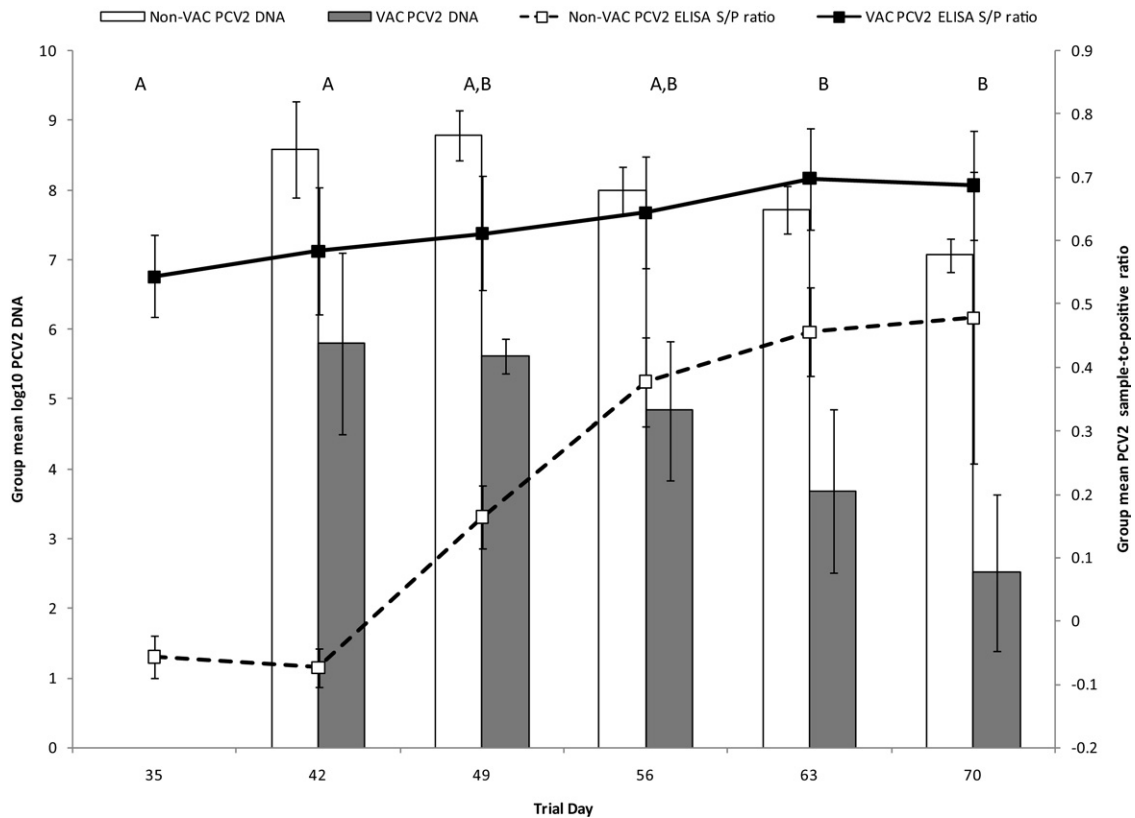


Fig. 2. Mean \log_{10} group PCV2 DNA amount and mean group PCV2 ELISA sample-to-positive (S/P) ratios for Days 35 (PCV2 inoculation), 42, 49, 56, 63, and 70 in serum samples from vaccinated (VAC-PCV2-I, VAC-MHYO-PCV2-CoI, $n = 6$) and nonvaccinated boars (PCV2-I, MHYO-PCV2-CoI, $n = 6$ on Day 42 and $n = 5$ on Days 49, 56, 63, and 70). An ELISA S/P ratio of ≥ 0.2 was considered positive. Days at which significant differences were observed between groups are indicated with and “A” (S/P ratio) or “B” (PCV2 DNA).

ratory distress, and mucopurulent nasal discharge. After PCV2 inoculation, one of the MHYO-PCV2-CoI boars became lethargic, lost body condition, had a bloody nasal discharge, and severe respiratory distress. This boar was given 3 mL of tulathromycin injectable solution (Draxxin®; Pfizer Animal Health, Inc.) i.m. on Day 49. However, this boar did not respond to treatment and died on Day 50.

3.2. Seroconversion to PCV2

On Day 0, all boars were negative for anti-PCV2 antibodies. Furthermore, based on weekly serology, none of the six sentinel negative control gilts seroconverted to PCV2 (data not shown). On Day 35, all VAC-PCV2-I and VAC-MHYO-PCV2-CoI boars had seroconverted to PCV2, with group mean S/P ratios of 0.49 ± 0.1 and 0.59 ± 0.01 , respectively, whereas PCV2-I and MHYO-PCV2-CoI boars remained seronegative. Overall, vaccinated boars had higher mean group S/P ratios on Days 35, 42, 49 and

56 compared to non-vaccinated boars (Fig. 2). Specifically, on Days 35 and 42, VAC-PCV2-I and VAC-MHYO-PCV2-CoI had higher ($P < 0.05$) group mean S/P ratios than non-vaccinated groups. Boars in the non-vaccinated groups seroconverted to PCV2 between Day 49 (PCV2-I, 2/3; MHYO-PCV2-CoI, 1/3) and Day 56 (PCV2-I, 3/3, MHYO-PCV2-CoI, 1/2). On Day 49, VAC-MHYO-PCV2-CoI had a higher ($P < 0.05$) group mean S/P ratio compared to the PCV2-I and MHYO-PCV2-CoI groups, and with no significant difference among groups on Days 56, 63, and 70.

3.3. Seroconversion to MHYO

On Days 0 and 35, all boars were negative for anti-MHYO antibodies. On Day 70, individual boars in the groups MHYO-PCV2-CoI (2/2) and VAC-MHYO-PCV2-CoI (2/3) had seroconverted to MHYO.

Table 2

Prevalence of PCV2 DNA positive pigs/total number of pigs in each group (mean amount of \log_{10} PCV2 DNA \pm SEM) in various sample types.

| Sample type | Group | Trial day* | | | | |
|-------------|-------------------|------------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| | | 42 | 49 | 56 | 63 | 70 |
| Blood swab | PCV2-I | 3/3 (7.4 \pm 0.5) | 3/3 (7.8 \pm 0.1) ^A | 3/3 (6.4 \pm 0.5) ^A | 3/3 (7.3 \pm 0.4) ^A | 3/3 (6.5 \pm 0.3) ^A |
| | MHYO-PCV2-CoI | 3/3 (7.6 \pm 1.7) | 3/3 (8.6 \pm 0.5) ^A | 2/2 (6.7 \pm 0.6) ^A | 2/2 (8.0 \pm 0.4) ^A | 2/2 (7.0 \pm 0.5) ^A |
| | VAC-PCV2-I | 2/3 (3.5 \pm 1.8) | 3/3 (5.5 \pm 0.4) ^B | 0/3 (0.0) ^B | 0/3 (0.0) ^B | 0/3 (0.0) ^B |
| | VAC-MHYO-PCV2-CoI | 2/3 (3.8 \pm 1.9) | 3/3 (6.0 \pm 0.3) ^B | 2/3 (3.6 \pm 1.8) ^B | 2/3 (3.7 \pm 1.9) ^B | 0/3 (0.0) ^B |
| Feces | PCV2-I | 2/3 (3.3 \pm 1.6) ^{A,B} | 3/3 (8.0 \pm 0.3) ^A | 3/3 (7.6 \pm 0.6) | 3/3 (7.1 \pm 0.1) ^A | 3/3 (7.2 \pm 0.1) ^A |
| | MHYO-PCV2-CoI | 3/3 (5.8 \pm 0.6) ^A | 3/3 (7.8 \pm 0.6) ^A | 2/2 (7.3 \pm 0.2) | 2/2 (7.9 \pm 0.3) ^A | 2/2 (6.0 \pm 1.0) ^A |
| | VAC-PCV2-I | 1/3 (1.6 \pm 1.6) ^{A,B} | 1/3 (1.9 \pm 1.9) ^B | 2/3 (3.5 \pm 1.8) | 0/3 (0.0) ^B | 0/3 (0.0) ^B |
| | VAC-MHYO-PCV2-CoI | 0/3 (0.0) ^B | 1/3 (1.6 \pm 1.6) ^B | 3/3 (5.8 \pm 0.3) | 1/3 (1.8 \pm 1.8) ^B | 0/3 (0.0) ^B |
| Semen | PCV2-I | 1/3 (1.8 \pm 1.8) | 3/3 (7.4 \pm 0.2) ^A | 3/3 (6.2 \pm 0.2) ^A | 3/3 (6.7 \pm 0.4) ^A | 3/3 (7.0 \pm 0.2) ^A |
| | MHYO-PCV2-CoI | 2/3 (4.8 \pm 2.5) | 2/2 (7.2 \pm 0.9) ^A | 2/2 (6.8 \pm 0.7) ^A | 2/2 (7.8 \pm 0.3) ^A | 2/2 (6.6 \pm 1.3) ^A |
| | VAC-PCV2-I | 0/3 (0.0) | 0/3 (0.0) ^B | 0/3 (0.0) ^B | 0/3 (0.0) ^B | 0/3 (0.0) ^B |
| | VAC-MHYO-PCV2-CoI | 1/3 (1.8 \pm 1.8) | 0/3 (0.0) ^B | 0/3 (0.0) ^B | 0/3 (0.0) ^B | 0/3 (0.0) ^B |

^{A,B} Within a column and sample type, means for amount of \log_{10} PCV2 DNA without a common superscript differed ($P < 0.05$).

3.4. Detection of PCV2 DNA

Based on weekly PCR testing of serum, all six sentinel negative control gilts remained negative for PCV2 DNA throughout the study (data not shown). On Days 49, 56, 63, and 70, vaccinated boars had less ($P < 0.05$) PCV2 DNA in serum compared to non-vaccinated boars (Fig. 2). The prevalence of PCV2 DNA positive blood swabs, fecal samples, and semen, and group means for amount of PCV2 DNA are summarized (Table 2). On Days 49, 56, 63, and 70, \log_{10} group mean PCV2 DNA levels were higher ($P < 0.05$) in non-vaccinated boars compared to vaccinated boars in all three sample types. For semen, only one of six vaccinated boars (VAC-MHYO-PCV2-CoI) was positive on Day 42, whereas non-vaccinated boars shed high levels of PCV2 DNA in semen on all days examined (42, 35, 49, 56, 63, and 70; Table 2).

3.5. Macroscopic lesions

The MHYO-PCV2-CoI boar that died on Day 50 had diffusely congested lungs with approximately 35% cranioventral dark purple consolidation (Fig. 3). This boar also had approximately 200 mL clear-yellow fluid in the thorax, and lymph nodes were approximately three times normal size. Macroscopic lesions in the remaining boars were limited to lung tissues, characterized by mild-to-moderate focal consolidations (ranged from 0 to 24% of the lung surface affected) in most individuals, regardless of infection or vaccination status.

3.6. Microscopic lesions

Microscopic lung lesions were characterized by mild-to-severe, multifocal-to-diffuse bronchointerstitial

pneumonia, with mild-to-moderate peribronchiolar lymphoid hyperplasia in MHYO-PCV2-CoI boars (Fig. 4). Lung lesions in all other boars were mild and appeared to be resolving. Boars in the PCV2-I and MHYO-PCV2-CoI groups had moderate to severe lymphoid depletion and histiocytic replacement of follicles in lymph nodes, spleen, and tonsil. One of three pigs in the VAC-PCV2-I and VAC-MHYO-CoI groups had focal (one lymph node affected), mild depletion of lymphoid follicles. There was mild interstitial edema in the testes of one MHYO-PCV2-CoI boar, but no remarkable lesions in the sex glands of any other boar. Porcine circovirus type 2 antigen was detected in lung tissues in 1/3 MHYO-PCV2-CoI boars (score 3, Fig. 5) and in lymphoid tissues of 2/3 PCV2-I boars (scores 1

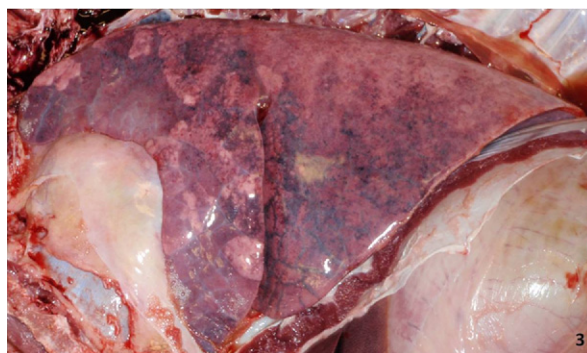


Fig. 3. Lung from a MHYO-PCV2-CoI boar that died on Day 50 (29 d after MHYO inoculation and 15 d after PCV2 inoculation). The lung failed to collapse; it had a diffuse mottled-tan appearance, with severe multifocal dark purple-to-tan consolidation, particularly in the cranioventral region.

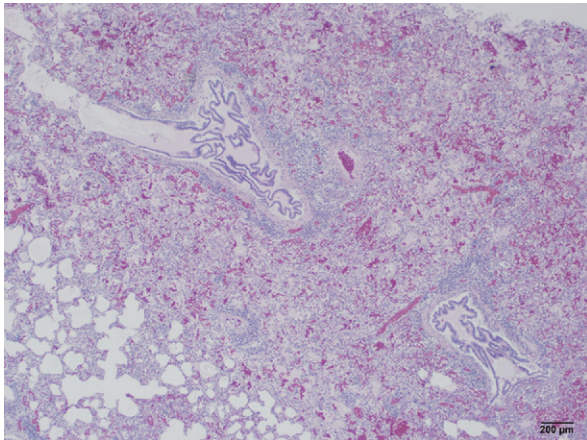


Fig. 4. Lung from a MHYO-PCV2-CoI boar that died on Day 50 (29 d after MHYO inoculation and 15 d after PCV2 inoculation). There was moderate peribronchiolar lymphoid hyperplasia (characteristic of MHYO infection), as well as mild alveolar edema and mild lymphohistiocytic interstitial pneumonia (hematoxylin and eosin stain).

and 2), 2/3 MHYO-PCV2-CoI boars (scores 2 and 3), and 1/3 VAC-MHYO-PCV2-CoI boars (score 1).

4. Discussion

In this study, clinical disease was reproduced in adult boars concurrently infected with MHYO and PCV2. Concurrent MHYO-PCV2 infection was associated with respiratory disease and infertility in an 11 month old boar from commercial boar stud in the USA, with PCV2 antigen detected within inflammatory cells in the interstitium of the seminal vesicles [33]. However, the present study was apparently the first time that PCVAD was reproduced in mature animals under experimental conditions. Boars coinfecting with MHYO and PCV2 developed moderate-to-severe clinical disease and 1/3 died, whereas 3/3 boars infected only with PCV2 remained clinically healthy. In addition, on Day 42, MHYO-PCV2-CoI boars shed more PCV2 DNA in semen than singularly infected pigs.

The negative status of the source herd and lack of cross-contamination between rooms in the research facility were assessed and confirmed with a group of sentinel gilts derived from the same herd and similar in age to the boars.

Differences in virus shedding between singular PCV2 and dual MHYO-PCV2 inoculated boars were not significant. A study with larger groups of boars is needed to conclusively determine the effect of concurrent PCV2 and MHYO infection. Only three boars were used in each treatment group in this experiment due to an extremely

limited supply of PCV2-naïve SPF boars. Furthermore, one of the MHYO-PCV2-CoI boars died prior to study termination further reducing the numbers of coinfecting boars. Notwithstanding, the trend was similar to previous observations in our growing pig model [24]. This further emphasized that common swine pathogens, generally regarded as being of low pathogenicity, can cause clinical disease when coinfecting.

Contaminated semen is a potential means of transmitting PCV2. Previous epidemiological investigations found no association between AI and occurrence of PCVAD [34,35]. However, AI has not completely been excluded as a potential point source of PCV2 infection, since the virus can be detected in semen by PCR [18–20,36] and virus isolation [36]. Naturally or experimentally infected boars typically shed low quantities of PCV2 DNA, either intermittently or continuously, with detection corresponding closely with viremia [18–22,36]. Although sows can be infected with PCV2 via AI using semen spiked with PCV2 inoculum [14], the definitive link between presence of PCV2 in boar semen and infection of breeding animals is still lacking. Nevertheless, the risk of transmitting PCV2 via semen and AI cannot be ignored, as PCV2 in semen from experimentally-infected boars was infectious in the swine bioassay [23] (although PCV2 quantities in semen were admittedly often low).

One of the objectives was to determine the magnitude of PCV2 shedding in semen under controlled conditions. Incoming boars are often exposed to multiple pathogens

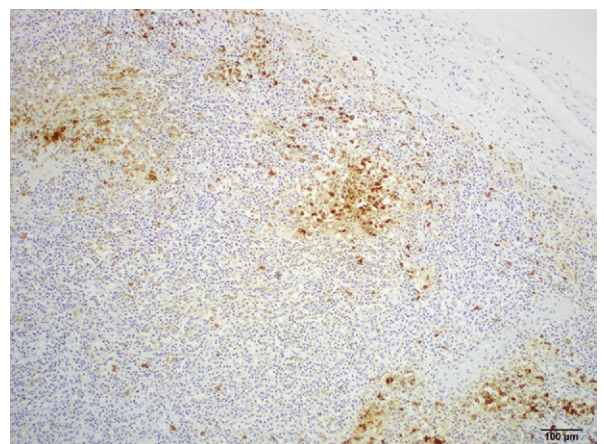


Fig. 5. Tracheobronchial lymph node from a MHYO-PCV2-CoI boar that died on Day 50 (29 d after MHYO inoculation and 15 d after PCV2 inoculation). Moderate-to large amounts of PCV2 antigen was present in several lymphoid follicles. Immunohistochemical staining with anti-PCV2 polyclonal antibody; streptavidin-biotin-peroxidase complex method, counterstained with hematoxylin.

and can be immune-stimulated with several adjuvanted bacterins. Immune stimulation in the form of commercial inactivated vaccination enhanced PCV2 viremia in growing pigs [30], with a more pronounced effect with specific adjuvants [37]. The PLE vaccine used in the present study is formulated with two adjuvants, including Amphigen®, the same adjuvant present in bacterins reported to enhance PCV2 replication [30,37]. Due to low numbers of available SPF boars, in this study all boars were vaccinated with the PLE vaccine and as such, the potential effect of PLE vaccination on PCV2 viremia and shedding was not determined. When compared with our previous study [22], using the same PCV2b isolate at a comparable infectious dose, the same route of inoculation, and a similar PCV2 detection method, the amounts of log₁₀ PCV2 DNA in semen 14 d post PCV2 inoculation ranged from 7.01 to 7.69 in PCV2b-infected and PLE-vaccinated boars, versus 4.43 to 4.93 in PCV2b-infected but non-vaccinated boars in our previous study (which corresponded to an approximately 2.67 log increase of PCV2 DNA load in semen). In the present study, the greatest amount of log₁₀ PCV2 DNA in semen was 8.09 (21 d post PCV2 inoculation in a MHYO-PCV2-CoI boar), whereas in the previous study, it was 6.15 (20 d post PCV2b infection in a PCV2 singular infected boar) [22].

Based on the growing pig model, PCV2 vaccination reduced viremia and shedding [38–40]. Moreover, PCV2 vaccination of pregnant animals prevented detectable PCV2 viremia in dams, and induced both serum neutralizing antibodies and colostral anti-PCV2 antibodies [41]. However, PCV2 DNA was detected in colostral samples and individual fetuses following oronasal PCV2 challenge [41]. A protective effect of PCV2 vaccination in boars has, to our knowledge, not been investigated under experimental conditions. In the present study, vaccination with a commercial PCV2 vaccine administered as a one-dose application resulted in a measurable anti-PCV2 IgG response at Day 35, and a significant reduction of PCV2 viremia and shedding after subsequent PCV2b challenge in mature boars. Under field conditions, piglets are often vaccinated against PCV2 at weaning to protect them from subsequently developing PCVAD. However, little attention has been given to adult animals, since they are not generally affected by PCVAD. In addition to conferring protection against clinical disease, a possible application of PCV2 vaccination in adult swine is reduction of PCV2 shedding and prevention of PCV2 spread to susceptible populations. In this study, vaccination with a PCV1-2a-based vaccine prevented not only clinical disease associated with concurrent MHYO-PCV2b in-

fection in boars, but also reduced PCV2b shedding in semen and feces, further confirming the cross-protective effect of the vaccine previously reported in growing pigs [38,40].

In this study, although boars experimentally co-infected with PCV2 and MHYO did not shed significantly more PCV2 in semen compared to those infected with PCV2 alone, coinfecting boars developed severe clinical PCVAD (which was fatal in one of three boars). In addition, PCV1-2a vaccination protected boars from developing clinical disease associated with PCV2b, and it significantly reduced PCV2 viremia and PCV2 shedding in semen. This work emphasized the clinical importance of PCV2 and MHYO coinfection in adult boars, and provided important information regarding the benefits of the PCV2 vaccine in the control of PCVAD and porcine respiratory disease complex in boar studs and breeding herds.

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